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Conceptual Developments in Photosynthesis, 1924–1974

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In constructing concepts about photosynthesis I shall use a historical approach. Since I am not by nature a historian, I have to disclaim any implication that the result will be a valid history. On the contrary, I shall present a highly editorial account based only on the qualifications that I have worked in the field for most of the period to be considered.

The concepts of photosynthesis of 50 years ago were summarized by the monographs of Spoehr (33) and Stiles (35). Spoehr was an organic chemist and his work was an American Chemical Society monograph; but only a small fraction of it would be recognized as chemistry by today's reader. The limitation was not imposed by the imagination of the scientists of that time. Spoehr devoted 30 pages to theories based on good, solid armchair chemistry. The limitation was simply in the lack of tools sharp enough to dissect further than the chlorophylls and the accumulated end products. It was necessary to treat the machinery as a black box and deduce its characteristics by measuring what went in and what came out.

There is little doubt that the greatest impact of the 1920's was made by Otto Warburg. However, it is a little surprising to consider that it was not biochemical; in fact, I shall develop the thesis that it was antibiochemical. But we should note first that Warburg's major contribution was technological rather than theoretical. He introduced, in 1919, (40) the manometric method for measuring gas exchange and a new experimental organism, *Chlorella*. Fifty-five years later both the method and the experimental organism are still in use. In order to appreciate the importance of that contribution it is necessary to consider the methods which had been in use for measuring the rate of photosynthesis.

The most informative methods were those which in some way measured gas exchange. They were macromethods having sensitivities measured in milliliters of gas. This meant large samples, leaves or whole plants, large areas to be illuminated, and rather lengthy experiments usually measured in hours. Warburg's new method suddenly increased sensitivity to measure microliters of gas exchanged and thereby decreased correspondingly the size of sample and the time of observation. Experimental material was an algal cell suspension which could be aliquoted. Sampling problems became minor. Smaller and more manageable light beams could be used.

I digress, to consider the important advance of the Warburg method in shortening the time base of experimental measurement. Subsequent technological advances have given far greater improvements in time resolution. We measure and distinguish biochemical events, faster photochemical events, and still faster photophysical events (Kamen's [26] time eras of photosynthesis). The Warbug method improved the time base from hours to minutes and thereby relieved us of the constraint of measuring only growth-bound or over-all metabolism.

If one seeks to understand a particular cellular process such as photosynthesis, only vague and uncertain information can be obtained from measurements of total or overall metabolism of the plant. All early studies of photosynthesis were subject to this serious constraint. In particular, consider early evidence for carbohydrate as the product of photosynthesis.

Early measurements of gas exchange on whole plants established an assimilatory quotient ($AQ = \Delta CO_2/\Delta O_2$) close to unity. The presumptive conclusion that carbohydrate is the product of photosynthesis was reinforced by observable accumulation of starch after long periods of illumination. No other possible conclusion could have been reached, for all measurements were made over periods so long that they described only over-all metabolism. The composition of higher plants predicts that the over-all metabolism must reflect their predominantly carbohydrate economy. The hallowed concept that carbohydrate is also the product of photosynthesis *per se* (whether or not a proper statement today) was only scientific mythology.

All of the above might be regarded as academic trivia until one considers the case of *Chlorella* and other algae in which gas exchange can be measured in short-time experiments. For many years *Chlorella* was thought of as simply a miniature of a higher plant. This was an error. *Chlorella* and other microalgae are microbes. Their metabolic business is the synthesis of new cells which are 50 to 60% protein and perhaps only 30% carbohydrate. Warburg's introduction of *Chlorella* is a curious anomaly. An essentially protein-synthesizing organism became a standard organism for studying photosynthesis, a process thought to be an exclusive synthesis of carbohydrate. If the original evidence for photosynthetic product had been obtained from long-time experiments on algae, instead of on higher plants, we would have started out with the conclusion that a major product is protein. Fortunately, it is rather easy to trick *Chlorella* into short periods of predominantly carbohydrate synthesis and this became a part of standard experimental technology.

A few of Warburg's other contributions (41–43) document his impact on the thinking of the 1920's. His very clean data on effects of temperature established the low Q_{10} (approximately 1.0) at low light intensity, the high Q_{10} ($\geqq 2$) at high intensity, and thereby confirmed the Blackman proposition that both light and dark reactions are involved. His flashing light experiments set the stage for the later Emerson and Arnold (10, 11) experiments. He observed the inhibitory effect of oxygen. This was not understandable biochemically until recently, even though Tamiya and Huzisige (37) later showed the interplay of O_2 and CO_2 concentration and concluded that the locus of effect was at the carboxylating enzyme. Warburg was not bound by the shibboleth that physiological conditions are needed to learn about physiology. By dropping the pH to 3, he obtained an unregulated nitrate reduction and demonstrated a photochemical reduction of nitrate and an equivalent production of ammonia. In retrospect, this might be viewed as an early demonstration of the Hill reaction. But the thinking of that time, least of all Warburg's, was unprepared for such an idea.

Certainly Warburg's most important conceptual contribution was in the measurement of quantum yield (43). Others had concerned themselves with energetics. Warburg counted quanta as befitted a photochemical process. He trained his *Chlorella* and arranged a protocol of measurement which gave him the most oxygen per quanta. What he sought was not an average value for all plants. He wanted to know how efficient the machinery could be. And he came to an experimental number of 4.3 quanta per oxygen. Of course, conceptually the quantum number became four, and as neat a number as one could hope for.

The interpretation of the four-quantum number which developed in the following years was equally neat and simple. The measured output was 112 kcal. The measured input of four quanta at 660 nm was 172 kcal. That left a thermodynamic loss for stabilization of intermediates at the uncomfortably low value of 60 kcal. Hence there was necessarily some unique photophysical mechanism which could carry carbon through its four reductive steps without leaving any spoor of stable intermediates. Certainly there was no room for biochemistry. The vision of Willstätter was sustained: the unique photochemical event must be performed upon a Chl-CO_2 complex.

The four-quantum dogma went unchallenged until the late 1930's. The first experimental challenge came from the Wisconsin group of Manning, Stauffer, Duggar, and Daniels (31). They used *Chlorella* in larger quantities and measured gas exchange by old-time volumetric methods over longer time periods. None of their quantum numbers approached four, and some were values in the hundreds; their estimate from one type of experiment was a quantum number of 20. A following flurry of quantum yield work persisted almost 20 years: was the quantum number low ($\leqq 4$) or was it high ($\geqq 8$)? The man-hours spent and the bitter controversy of symposia must have been a source of wonderment to the rest of the scientific world. The argument was not over until Warburg's death. But reason for concern about the quantum number had then disappeared. The demonstrated biochemical and biophysical events required energy losses which could not possibly be driven by four quanta per O_2.

A salient event of the early 1930's was van Niel's theory which I shall consider as set forth in 1935 (38). Detailed critiques (15, 34) have been made. In Gaffron's words, "purple bacteria furnished van Niel the key to the first generally convincing picture of the photosynthetic process in terms of modern metabolic ideas." There were three initial steps in van Niel's argument.

1. The green and purple sulfur bacteria perform a reduction of CO_2 to organic matter which is dependent upon illumination. Therefore they perform a photosynthesis.

2. The metabolic processes of bacteria are best understood as a series of successive dehydrogenation reactions, $H_2A + B \rightarrow A + H_2B$.

3. There is a formal homology between bacterial and green plant photosynthesis:

$$CO_2 + 2H_2A \xrightarrow{\text{light}} (CH_2O) + H_2O + 2A.$$

This generalized equation implies that "for different photosynthetically active organisms different hydrogen donors for the final reduction of CO_2 may be required."

The compulsions of comparative biochemistry forced van Niel to a fourth step in his argument. In the various photosyntheses describable by a generalized over-all equation, there ought to be some common denominator. CO_2 reduction did not seem very special to a microbiologist. There were known autotrophic bacteria which accomplished that in the dark, so he proposed that in all photosyntheses the photochemical event occurs at a Chl·H_2O complex providing an (H) for CO_2 reduction and leaving an oxidized moiety. Differences in the photosyntheses arise from the H-donor (*e.g.*, H_2O, H_2S, organic substrate) used to remove that oxidized moiety.

Gaffron (14) showed that one also needed to think of metabolic-like donors even if the photochemical event were performed on a Chl·CO_2 complex. But he also went on to achieve "an experimental modification of the behavior of green algae so that photosynthetically they have become purple bacteria" (39). Gaffron rather quickly accepted the logical force of van Niel's fourth proposition and thereby spared a polemic which, in retrospect, would have been rather useless. Even though the generality of the proposition turned out to be wrong, we now had the words metabolic and H-donors in the lingo of photosynthesis. And we had the right to think of water as being close to the photochemical event.

The second great event of the 1930's was the work of Emerson and Arnold (10, 11). These experiments are great ones in design, in technical accomplishment, and in conceptual value of the end result. If photosynthesis requires both fast photochemical reactions and slow enzymatic reactions, and if these operate cyclically and in series, then it ought to be possible to separate them in time. One must use flashes bright enough that all photochemically active units will work during the flash, short enough that no photochemically active unit can work more than once during a flash, and separated by dark periods long enough to complete the cycle of necessary enzymatic reactions. From their first paper Emerson and Arnold reasonably believed that these requirements were experimentally attained. The maximum flash yield (actually summed for several thousands of flashes) was temperature independent. Only the dark time required to obtain maximum flash yield, presumably the time for the Blackman enzymatic reactions, was temperature dependent.

It was the second paper which proved upsetting to all previous thinking. In a sample of *Chlorella* which produced n molecules of O_2 per flash, the number of Chl molecules was about 2000 n. Evidently there was a mechanism by which 2000 Chl could cooperate in the reduction of one CO_2.

I will not belabor the early tortuous history of the concept of the photosynthetic unit. Objections to it were partly technical (were the flashes really saturating?), partly raised by other nonconfirming experiments (usually with longer flashes), partly because it seemed possible to find explanations less astonishing than a cooperative assemblage of Chl molecules. Photosynthesis had not yet escaped the clutches of solution photochemistry.

A third significant advance of the 1930's was the development of photoelectric spectrophotometry (23) and a renewed development of chromatography (45) for purification of plant pigments. It became possible to make rapid and quantitative analysis for Chl *a* and *b* and the other plant pigments (7).

It was in the early 1940's that it became legitimate to think of photosynthesis as a photolysis of water. One evidence came from observations that "the O^{18}/O^{16} ratio of the evolved oxygen is identical with that of the water" (32). The original data were very clean and left little margin for error but subsequent re-evaluation was called for by the fact that, as compared to H_2O, both carbonates and atmospheric oxygen have a measurably higher O^{18}/O^{16} ratio. By 1945 re-evaluation had affirmed the high probability of correctness in the original interpretation, and left the conclusion that there must be other (second order) exchange reactions which give rise to enrichment of O^{18} in the atmosphere.

The isotope ratio data gave very high probability to the idea that O_2 evolved in photosynthesis derives from H_2O (rather than from CO_2). It did not tell about mechanism of O_2 produc-

tion (which is still unknown). It did not require, but it made acceptable, the supposition that the $H_2O \rightarrow O_2$ conversion was close to the photochemical reaction. This possibility became more plausible from the concurrent work of Hill. However, it was not immediately obvious that this was so: neither Kamen nor Hill referred to the work of the other although their papers overlapped in time.

The four papers of R. Hill (17, 18, 20, 21), over the three years 1937–1940 (the latter two with R. Scarisbrick), provide a case history of discovery which any scientist might ponder. Chloroplasts were the evident organelles for photosynthesis: they contained the pigments and they accumulated starch in light. If chloroplasts were isolated from a cell, the transient production of a little oxygen could be detected, but not measured. Hill recognized the high affinity of muscle hemoglobin as the basis for a very sensitive measurement of oxygen.

In his first reported experiment, Hill (17) measured the time course of O_2 production by chloroplasts of *Lamium album* over 2 minutes. By that time total O_2 production had reached 0.3 μl. To get even that much O_2 he had had to add back a leaf extract. Ferric oxalate proved to be equally as good as leaf extract. The questioning experiments had been done. The answer was positive. But Hill was reserved in his conclusion about relationships to photosynthesis. He did recognize that isolated chloroplasts provided "new possibilities for applying biochemical methods to the green plant."

Three years and two papers later, after some very thoughtful experiments, Hill and Scarisbrick (20) allowed themselves the conclusion that "the light reaction in vegetable photosynthesis is the production of the oxygen molecule and is not the reduction of carbon dioxide." And then they went on to show that, with added ferricyanide as the ultimate acceptor (not reoxidized by O_2), they could demonstrate significant rates and quantities of O_2 by conventional manometric methods. It might seem that by this time the chloroplast would have become as visible to the physiologist as it had been to the microscopist. But chloroplasts were not yet glowing and visibility came slowly.

In 1941 I had the privilege of reading on behalf of French and Anson the first paper before this society on the phenomenon which they properly called the Hill reaction. It was greeted by a rather stony silence except for questions about possible direct photodecomposition of iron salts. It took a while for us to build into our imaginations the significance of the Hill reaction.

I have made a production out of Hill's work. From it one can trace multiple lines of thought and experiment. First, there is the strong conceptual base for the Hill reaction as a photolysis of water. Today we cannot say that water-splitting is a primary photochemical event, but that is because our time resolution of events has improved. Second, there is a line of work which arose from asking about other substitute oxidants which could serve (instead of CO_2) for the Hill reaction. Although this line remains open ended, it led rather rapidly from Fe^{3+} to NADP. Third, and still more important, Hill dissected out the working chloroplast and provided experimental material for biochemical and further physical dissection.

The 1940's saw the emergence of a third concept which can be traced through current work, *viz.* fluorescence of Chl *in vivo* contains information about photochemical events in photosynthesis. The first argument was strictly *a priori:* a Chl molecule cannot use the same quantum of energy for both fluorescence and photochemistry. A second argument came from simultaneous measurements of fluorescence and CO_2 uptake during the induction period. There it was easy to demonstrate an obvious correlation between fluorescence yield and rate of photosynthesis. So there was little doubt about the underlying concept. The uncertainty lay in interpretation (and still does, at least quantitatively). Of the several positions taken, two had impact.

The Utrecht Biophysical Group, arguing first from effects of H-donor limitations on fluorescence in purple bacteria, came to a simple proposition: fluorescence is "an energy flow meter," an (inverse) "measure of the transfer of energy to the photosynthetic energy acceptor" (27). James Franck (13) took a very different position. He believed steadfastly in a photochemical event at a Chl-CO_2 complex. He was impressed by the relatively prompt effect of CO_2 on fluorescence, the effects of anaerobiosis, the problem of preventing Chl photooxidations, and the increase in fluorescence yield caused by narcotics and light saturation. He was led logically to an interpretation of fluorescence changes caused by accumulation of natural narcotics (plant acids) on the Chl surface.

I have belabored Franck's contribution, of which almost nothing remains today, simply because he was the acknowledged theoretician of photosynthesis for many years. I knew James Franck, in the way that a graduate student might know a great scientist of another university. I admired his intellect, his force, and his personal charm. It is not my wont to be ungracious. But in a completely dispassionate way, I have had to wonder why it was that Franck made so little impact now discernible in development of present concepts. I find one reasonable clue. Franck believed that a satisfactory theory must be all-embracing, "must be in agreement with all available evidence." Not all of the published data about photosynthesis can possibly mean what we think it means. An all-embracing theory on a problem so complex must contain assumptions as *ad hoc* as Franck's plant acid narcotics and his "catalysts A, B and C." The theory was so broad as to be almost unassailable and untestable. It did not suggest what the next experiment should be and thereby was almost sterile.

In the 1950's, the structures of three important concepts were defined. First, there was the mechanism of carbon dioxide fixation and reduction in what came to be called the Calvin-Benson cycle. This was the result of great labor and skill applied over a number of years in a team effort. The first result (unexpected at the time) was the early labeling of carbon from $C^{14}O_2$ in many different small molecules. Then came the decisive selection of phosphoglycerate as the first fixed product, and finally ribulose diphosphate as the CO_2 acceptor (4). If one wishes to view photosynthesis in terms of carbon metabolism then a great deal more should be said. If one views the reduction of carbon dioxide as a single category of events, as I do, then a simple statement summarizes the conceptual contribution of Calvin and his colleagues: the energetic requirements of carbon dioxide reduction can be provided by NADPH and ATP. This concept (even including the inferred 2 NADPH:3 ATP:1 CO_2, stoichiometry) has become dogma, embellished to the tacit assumption that the only interplay between carbon metabolism and the rest of photosynthesis occurs in terms of NADPH and ATP. Really, we should give some thought also to the H^+ ion demands and their contribution to ion gradients. But beyond this, I think we should label the assumption more clearly. The history of science shows that a tacit assumption may be a dangerous assumption.

A second accomplishment of the 1950's was Arnon's demonstration of "the chloroplast as a complete photosynthetic unit" (3), in the sense that the chloroplast can accomplish oxygen evolution from water, phosphorylations, and reduction of carbon dioxide to stable products. The concept emerged from a great deal of work, not all from a single laboratory, most of it devoted to the operational description of cyclic and

noncyclic phosphorylation. In the chloroplast, we now had all that Hill's original intuition could possibly have imagined. In photophosphorylation we had a common denominator which van Niel had sought for in green plant and bacterial photosynthesis.

A third important accomplishment of the 1950's was a roughed-out picture of the physical events which precede (what we loosely call) photochemistry. Action and quantum yield spectra for oxygen evolution had demonstrated that light absorbed by accessory pigments (Chl *b*, phycocyanin, phycoerythrin, and fucoxanthin) can run photosynthesis at close to maximum efficiency. Action (or excitation) spectra for Chl *a* fluorescence corresponded closely to action spectra for oxygen evolution. The remarkable dissertation of Duysens (8) put all this into an hypothesis phrased in a language which we still use. A rapid and efficient transfer of excitation occurs by inductive resonance from the accessory pigments to Chl *a*. Further transfer proceeds with (potentially) high probability to a reaction center, a special form of Chl present in small concentration.

The reaction center therefore becomes the guts of a photosynthetic unit. On one side it looks out upon many light-harvesting molecules of its pigment system where lifetimes of excited states are measured in nanoseconds or less. On the other side, it provides the more stable oxidized and reducing moieties, with lifetimes measured in milliseconds, accessible to metabolic events.

Duysen's work encompassed the photosynthetic bacteria. He also demonstrated the energy transfer of the several bacterial Chl B 800 → B 850 → B 890 and an absorption change at 890 nm attributable to reaction centers. The idea of pigment systems and reaction centers became a second common denominator of bacterial and green plant photosynthesis.

A notable event of the 1950's was the discovery of delayed light (36), providing evidence for some kind of energy storage capable of returning Chl molecules to the excited singlet state. The phenomenon is less than completely understandable today, though it has been widely used as a tool. Perhaps its major impact, developed by Arnold (2), is that some of the events in a photosynthetic membrane are describable in terms of the electrons and holes of solid state physics.

The late 1950's saw the accumulation of anomalies which, in the end, gave rise to recognition of two photoreactions. This became a transition in thought so drastic that one can speak of the 1960's as the beginning of a modern era in photosynthesis. It is useful to ask why was that transition so drastic and traumatic? My own answer also contains a lesson. It had been perfectly sensible to follow the assumption of a single kind of photochemical event. That was the simplest possible assumption. Where we had erred was in not recognizing, in leaving tacit, that assumption.

I purposefully shall dwell on the first recognized anomaly. At the 1957 Gatlinburg Conference, Blinks reported his observation of chromatic transients (5). He had quickly shifted his monochromator between two wavelengths which happened to give equal rates of oxygen evolution. Immediately after the wavelength shift, there were marked transients in rate of oxygen evolution. Today the observation can be considered an entirely expected result of the characteristic of two light reactions. The first report was greeted by that evidence of disbelief *viz.* search for trivial explanation. Naturally Blinks himself had no sensible explanation other than that it might be a respiratory artifact.

A second anomaly was Emerson's (12) enhancement phenomenon. This one was more evident because it dealt with the more familiar quantum yield. At long wavelength the unexplained drop in quantum yield was (at least partially) prevented

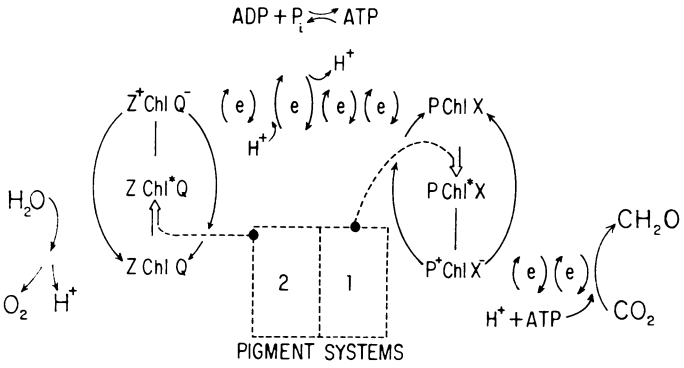

FIG. 1. Events in photosynthesis in the context of the Z-scheme and as a framework for discussion. Its incompleteness and some possible errors are considered in the text.

by a supplemental light of properly chosen wavelength. And the best choice of wavelength for supplemental light was one at which there was a high fractional absorption by an accessory pigment.

A third anomaly arose in the behavior of P_{700}. The discovery of P_{700} in itself had been an achievement notable in conceptual design and experimental development. Kok (29) had set out to find the ultimate photoreceptor of photosynthesis, hopefully to be recognized by a small absorption change on the long wavelength side of the large *in vivo* Chl absorption band. Anyone who has measured P_{700} by our current and abridged methods cannot help but be impressed by the experimental sophistication which led to Kok's demonstration of P_{700} as (at least a part of) the reaction center for photoreaction 1. The anomaly provided by P_{700} was that far red light bleached it, while red light (<670 nm) restored absorption. This led to the first proposal, explicit in terms of separate quantum yields and rate constants, for two separate light reactions (29).

A fourth anomaly involved the Cyt and developed along two different converging attacks. Hill's laboratory had demonstrated two chloroplast Cyt, *f* and b_6, which appeared similar to mitochondrial Cyt *c* and *b*. In search of a function for Cyt *f* and b_6, with potentials of about 0.37 and 0 v, Hill and Bendall (19) were led to suppose a minimum of two light reactions separated by an exergonic electron transfer. By putting this magnificent speculation on a potential diagram they gave birth to the Z-scheme (Fig. 1).

In a second attack Duysens (9) had been searching for *in vivo* absorption changes at 420 nm and 550 nm ascribable to Cyt *f*. Light-minus-dark difference spectra showed the expected bands as an oxidation of the Cyt by white light. With monochromatic actinic lights a new characteristic emerged. In *Porphyridium*, 680 nm of light rapidly oxidized, but an added 562 nm of light partially reduced, Cyt *f* as evidenced by its 420-nm absorption band. Added DCMU blocked the reducing effect of 562 nm. Cyt *f* oxidation was thereby ascribed to a photosystem 1 and its reduction to a photosystem 2.

By the early 1960's, the accumulated anomalies had forced the acceptance of the necessity for two light reactions, photoreactions 1 and 2 occurring at different reaction centers, and pigment systems 1 and 2 as their corresponding pigment antennae. Presumptive evidence was at hand for a series arrangement of the two photoreactions and their interaction in terms of electron transport between them, *viz.* the Z-scheme. Alternative possibilities were envisioned and developed to explain various localized observations. But no alternative hypothesis accommodated so many different kinds of observations. Additional

components such as the plastoquinones, plastocyanin, and ferredoxin were fitted to the scheme.

The essential argument for the Z-scheme has been developed by evidence that (at least a major fraction of) electron flow in photosynthesis occurs between the light-reducible side (Q) of the system 2 reaction center to the light-oxidizable side of the system 1 reaction center. Most of the evidence has been obtained by perturbing the system in some way, by a light flash or wavelength change, and observing the kinetics of return to a chosen steady state (*e.g.* 1, 25, 30, 44). Out of this has come information on pool sizes of intermediates, their apparent sequence, and rate constants. It is noteworthy that the limiting rate constant of about 100/sec (referenced to Q, 25 C) roughly corresponds to the long ago estimated maximum turnover rate for a photosynthetic unit. Internal consistency is provided by reasonable correspondence obtained from observations of O_2 evolution (beyond the oxidized side of center 2), variable fluorescence (reduced side of center 2), P_{700} (oxidized side of center 1), and viologen dye reduction (beyond the reduced side of center 1).

The Z-scheme has been embraced avidly by the textbook writers as a graphic framework used to show how real-life photosynthesis works. I prefer to think of it as a working hypothesis which is supported by or consistent with most (but not all) pertinent data. It is manifestly incomplete in at least two important ways. We have no hard information on (*a*) the arrangement and relative numbers of the two kinds of reaction centers, or (*b*) the stoichiometry between the two light reactions. (Kinetic models assume a 1:1 stoichiometry, only for convenience.) Even at best, the Z-scheme is a partial or limited hypothesis since it really concerns interaction between the two photoreactions at a metabolic (or electron transport) level. I will call it the first hypothesis.

A second hypothesis is needed to explain the events which precede the photoreactions: how is excitation energy delivered to two different reaction centers in such a way as to run the total system at maximum and rather high quantum yield? The simplest idea (9) is that of two rigid or separately packaged pigment systems. But there are sufficient anomalies that one can be less than satisfied. There has remained a recognized question whether there may be a spillover of excitation energy from pigment system 2 to system 1. Perhaps it might be better to question whether pigment systems 1 and 2 (those pigment molecules rigidly bound to their respective reaction centers) really include all the light-harvesting pigment molecules. Even on the question of the architecture of pigments and reaction centers we do not have hard information. We do not know whether reaction centers are arranged in a continuous bed of a pigment system (giving only statistical meaning to a photosynthetic unit) or whether they are arranged with their pigments in discrete (walled-off) units. In short, the second hypothesis is firm in the concept of migration of energy among pigment molecules and trapping by reaction centers but it is soft on the details of traffic control.

A third hypothesis is needed to understand the unique events which occur at the reaction centers. Both centers are usually pictured as complexes at which electronic charges may be physically separated and made accessible to metabolic electron carriers. Thus, center 1 has been postulated as undergoing the reaction

$$P_{430} \cdot P_{700} \xrightarrow{h\nu} P_{430}^{-} \cdot P_{700}^{+},$$

in which the two moieties are distinguished by absorption changes at the designated wavelengths (22) and P_{700}^{+} is also observable by electron paramagnetic spectroscopy. Center 2 has been postulated as undergoing the reactions

$$\text{Cyt } b_{559} \cdot \text{Chl}_{680} \cdot C_{550} \xrightarrow{h\nu} \text{Cyt } b_{559} \cdot \text{Chl}_{680}^{+} \cdot C_{550}^{-} \rightarrow \text{Cyt } b_{559}^{+} \cdot \text{Chl}_{680} \cdot C_{550}^{-}$$

implying a redistribution of charge after the light event (6). These examples are cited only to show current thinking. Actually, the more easily isolatable bacterial reaction centers are more completely described and are more likely to be understood first.

A fourth hypothesis is needed to explain the events leading to O_2 evolution. Biochemically, this has been developed up to the point of assigning the Mn requirement to a site close to the point of oxygen liberation and close to the photoreaction 2 center. Biophysically, the mechanism can be described by a model requiring a linear sequence of charge accumulation on the oxidizing side of the reaction center, as: $S^0 \rightarrow S^{+1} \rightarrow S^{+2} \rightarrow S^{+3} \rightarrow S^0 + O_2$ (28). Discovery was made possible by a fortuitous characteristic of the system, *viz.* back reactions occur slowly (sec) leaving most centers in the S^{+1} state. Likewise, the forward reactions seem to contain thermal events, albeit rapid ones with time constants close to 1000/sec. These details are cited for two reasons. The slow back reactions require that the classical light intensity curve of photosynthesis is really nonlinear at very low intensities (a characteristic readily demonstrable once the phenomenon is known). The fast forward reaction means that the intuitions of van Niel and Hill were indeed correct: the oxidation of water really is very close to the light event. The chemical details of O_2 evolution remain unknown and are a candidate for another unique event of photosynthesis.

A fifth hypothesis is needed to account for the phosphorylations. The concept of cyclic and noncyclic as two distinguishable types of photophosphorylations has survived for some 15 years. Unfortunately, neither stoichiometry nor rates of *in vivo* photophosphorylations have been made clear. A common question has to do with sites of phosphorylation but "site" seems to have developed a special meaning: the locus of electron transport needed to power the event. The problem of photophosphorylation, like that of oxidative phosphorylation, has become a central problem of biochemistry. We are aware of a complex of events necessarily occurring on membranes, *e.g.* conformational changes, membrane potentials, H^+ and other ion gradients, and an isolatable coupling factor. The phenomena are plentiful; it is the chain of cause and effect which is unclear. Until the details of membrane events surrounding phosphorylation are known, we shall have necessary uncertainties in other areas of hypotheses about electron transport and possibly about events at reaction centers.

On the problem of phosphorylation I have little expertise and so resort to the philosophical kind of observation usually condoned in senior citizens. I note that biochemists and photosynthologists have shown about equal forgetfulness. When biochemical fashion changed from the old Wieland (H) transfer to the more appropriate (H^+ + e) transfer, it seems that everyone turned to e transfer and almost forgot about the H^+. In photosynthesis, we thought carefully about the problem of moving electrons from H_2O to CO_2 and forgot about the problem of moving H^+. Certainly H^+ must be exported by the H_2O-oxidizing site and consumed at the CO_2-reducing site. These two sites certainly had to be separated, likely by a membrane, and there was evidence of all the requirements for a possible proton pump. The details of my model may be frowned upon but the basic idea is too obvious to be ignored. Actually, it took the surprising experiments of Jagendorf and his colleagues (24) to demonstrate that proton gradients are

produced in chloroplasts and, when generated artificially, can give rise to phosphorylation. It would seem rather foolish to spend the necessary extra energy in electron transport needed to produce a proton gradient without finding a way to use it. I claim no novelty for this simple-minded and partly teleological harangue; it just seemed that it should be said out loud.

A sixth hypothesis is needed to explain the sequence by which reducing power and ATP are stabilized by useful organic syntheses. In the late 1950's the hypothesis of the pentose cycle seemed satisfactory in the sense that anomalies were relatively few. Anomalies began to accumulate. Questions were being asked again about the real life photosynthesis of higher plants, a subject almost neglected for 50 years. Different species of higher plants differed markedly in their CO_2 compensation points. A distinguishable photorespiration became evident as a partial recycling of carbon in light. One of the most productive crop plants, sugar cane, showed remarkably fast incorporation of C^{14} from CO_2 into malic and aspartic acids. Such divergent observations as leaf anatomy and the C^{13}/C^{12} ratio got into the act. It became clear that plants are not all alike in their pathways of carbon fixation, currently classified as pentose, C_4, and crassulacean acid metabolism. In overly brief summary: it is evident that plants have been far more inventive than we once thought in responding to the low CO_2, the high O_2, the water stresses, and temperatures of their environment. It may well turn out that biochemical strategies for collecting, concentrating, and fixing C are as elegant as the biophysical strategy for collecting, concentrating, and converting light energy.

The latter part of this discussion I have framed in terms of a series of hypotheses. I wanted to show that what we have been doing is a dissection or resolution of the total problem of photosynthesis into separable points of attack. This is in sharp contrast to, and a long way ahead of, the early attempts at all-embracing theories. I have also tried to distinguish at least some of the places where our hypotheses are soft and our ignorance greater. For it is ignorance, rather than knowledge, which drives us (16). In trying to formulate discrete partial hypotheses there are also recognized dangers. I certainly am subject to criticism in the sense that no two people in the field are likely to choose the same degree of resolution for different parts of the total system. In a more general sense there is a danger that discrete partial hypotheses must also imply unit processes. We are well aware of the mistakes that can be made in biological systems by applying the unit process approach of the chemical engineer.

The earlier part of this discussion considered concepts, some which survived, some which failed. Of those that survived some were faulty or downright erroneous in their original context. Of those that failed, some were at one time widely accepted. One reaches the conclusion that some of the concepts we cherish today will perish tomorrow. I find no comfort in the common statement often found in the introduction of a current paper: "It is generally accepted that. . . ." I have not referred to some bloody battles, or at least high adrenalin debates, over what should be "generally accepted." They are notable today only for their futility. The test of a concept, like the question of pregnancy in the human female, is not current majority opinion but the test of time.

I have written too many words. This thought leads me to reflect on a postulate of William Arnold who has followed the development of photosynthesis longer than I. In tribute I quote an Arnoldism: A scientist should be required to present his work by carving his words in stone. Then he would use few words and he would make very sure that he was right.

Note Added in Proof. On rereading for proof I am appalled at the many obvious omissions. Citation of a few key papers allows development of concepts but does not do justice to critical supporting contributions and parallel discoveries. One particular omission should be noted. Eugene Rabinowitch provided the 2000 page monograph, "Photosynthesis and Related Processes," which added his own insight to an encyclopedic coverage of the field to 1955. He was what every scientific area of endeavor needs: a broadly knowledgeable and incisive critic.

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